APPLICATION OF BACTERIAL LIGNINASE ENZYMES FROM *RYNCHOPHORUS FERRUGINEUS'S* GUT CONSORTIUM IN AGROPULP BIODELIGNIFICATION

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DEDICATION

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ABSTRACT

Lignin is a complex phenylpropanoid heteropolymer entangled around cellulose and hemicellulose fibre. It is recalcitrant towards degradation, thus pose a major problem in obtaining good quality fibre. Moreover, the conventional degradation methods (chemically and mechanically) consume vast amount of chemicals and energy which have deleterious effects to the environment. The aim of this research is to identify an alternative process for lignin removal by employing ligninase produced by lignin-depolymerising bacteria isolated within the gut of wood feeding insect, *Rynchophorus ferrugineus*. In this study, four bacterial species capable of depolymerising lignin were isolated, identified and characterised as *Klebsiella pneumoniae, Pseudomonas citronellolis, Enterobacter oryzae,* and *Serratia mercescens*. The production of ligninase enzymes (lignin peroxidase, manganese peroxidase and laccase) in all species were optimised using response surface methodology (RSM). The results revealed maximal production of ligninase is achieved at optimal pH of 5 to 6 and optimal temperature of 40 °C after 7 days of incubation. The ability of each specie to remove lignin in agrowastes (cogon grass (CG) and oil palm leaves (OPL)) was validated through UV-Vis spectrophotometer, FTIR and SEM analyses. The results showed that the gut microbes were solely able to degrade lignin with 30 to 60% in OPL and CG respectively, in which the highest delignification is obtained by the combination of all species. Furthermore, traces of small molecular weight compounds generated after the enzymatic breakdowns were identified through HPLC and GC-MS analysis as part of the complementary study. Through the identification of metabolites produced, the conversion pathways of lignin polymer to various aromatic and non-aromatic compounds during the breakdown were also proposed. The findings of this research can be made applicable to the pulp and paper industries thus encouraging the use of green and sustainable technology. The identification of breakdown metabolites could be exploited in the valorization of lignin promoting the concept of ‘waste to wealth’.

ABSTRAK

Lignin adalah heteropolimer *phenylpropanoid* yang kompleks tersusun sekitar selulosa dan hemiselulosa. Lignin mempunyai daya tahan tinggi terhadap degradasi seterusnya menghalang proses penghasilan serat yang berkualiti. Selain itu, kaedah konvensional (kimia dan mekanikal) untuk memisahkan serat dari sebatian lignin menggunakan sejumlah besar bahan kimia dan tenaga serta memberi impak negatif terhadap alam sekitar. Tujuan penyelidikan ini dilakukan adalah untuk mengenalpasti proses alternatif bagi penyingkiran lignin dengan menggunakan enzim (*ligninase*) yang dihasilkan oleh bakteria yang terdapat di dalam usus *Rynchophorus ferrugineus*. Hasil kajian menunjukkan terdapat empat spesies bakteria yang mampu mengurai lignin yang dikenali sebagai *Klebsiella pneumoniae*, *Pseudomonas citronellolis*, *Enterobacter oryzae*, dan *Serratia mercescens*. Penghasilan enzim *ligninase* (peroksida dan *laccase*) oleh semua spesis telah di optimumkan menggunakan *response surface methodology* (RSM). Penghasilan maksima bagi enzim *ligninase* dicapai pada pH optimum iaitu 5 hingga 6 dan suhu optima 40 °C selepas 7 hari inkubasi. Keupayaan setiap spesies untuk menyingkirkan lignin dalam bahan sisa agro (cogon grass (CG) dan daun kelapa sawit (OPL)) telah disahkan melalui analisis spektrofotometer UV-Vis, analisis FTIR dan SEM. Hasilnya, mikrob usus dapat menyingkirkan lignin dalam 30 hingga 60% dalam OPL dan CG, dengan peratusan tertinggi diperolehi oleh gabungan spesies. Tambahan lagi, kompaun molekul kecil yang dihasilkan selepas rawatan enzimatik dikenal pasti melalui analisis HPLC dan GC-MS sebagai sebahagian daripada kajian pelengkap. Melalui produk metabolit yang terhasil, laluan penukaran polimer lignin kepada sebatian aromatik dan bukan aromatik semasa proses penguraian dikenalpasti. Penemuan penyelidikan ini boleh digunakan untuk industri pulpa dan kertas serta menggalakkan penggunaan teknologi hijau dan lestari. Selain itu, produk metabolit boleh dieksploitasi dalam meningkatkan nilai lignin yang dapat mempromosikan konsep 'waste to wealth'.

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LIST OF SYMBOLS AND ABBREVIATIONS

3D	-	Three dimensional
ABTS	-	2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
BLAST	-	Basic Local Alignment Search Tool
BSA	-	Bovine serum albumin
COMT	-	Caffeic acid 3-O-methyltransferase
C=C	-	Carbon to carbon bonding
C=O	-	Ether bond
Ca^{2+}	-	Calcium ion
$Ca(OH)_2$	-	Calcium hydroxide
CaCl	-	Calcium chloride
ddH_2O	-	Distilled water
DNA	-	Deoxyribonucleic acid
DyPs	-	Dye-decolorizing peroxidase
EPA	-	Environmental protection agency
FTIR	-	Fourier-transform infrared spectroscopy
GC-MS	-	Gas chromatography – mass spectrometry
GHG	-	Greenhouse gasses emission
HAP	-	Hazardous air pollutants (HAP)
H_2O_2	-	Hydrogen peroxidase
HPLC	-	High pressure liquid chromatography
H_2SO_4	-	Sulphuric acid
$KHPO_4.7H_2O$	-	Potassium hydrophosphate heptahydrate
IPCC	-	Intergovernmental Panel on Climate Change
Lac	-	Laccases
LB	-	Luria broth

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